DECLARATION UNDER 37 C.F.R. 1.132

Dear Sir:

Juan Ramón CONDE RUIZ, a citizen of Spain, having a residence at c/ Sirio nº 50, 28007-Madrid (Spain), declare as follows:

1. That I am a graduate in Pharmacy in the University of Granada (1957-1963). From 1966 to 1968, I worked in the Research Laboratories, Department of Pharmacology and Toxicology of CERM, a pharmaceutical Company in Clermont-Ferrand (France).

I have been assistant lecturer for Pharmacology at the University of Granada (1970-1973).

I am founder and manager for thirteen years of the Center of Pharmacological Research of Liade S.A. Laboratories (1968-1981).

At present I collaborate with the Pharmacology Department of the Complutense University of Madrid in the doctorate subjects.

2. Since 1983, I am as responsible of the Pharmacology Department of ELMU, S.A. I have developed the pharmacological and toxicological tests related to Fepradinol, Lactalfate and the topical corticosteroids programmes, with especial reference to Ciclesonide.

I have been founder member of numerous spanish and foreigner professional societies. Member of the Laboratory Animals Protection Society. I have attended numerous

congresses, symposiums and sound round-table conferences. I am titular of numerous patents both in Spain and in many countries.

At present, I am speaker in conferences about GLP's introduction in Spain.

I am the author and coauthor of numerous scientific publications and communications related with antiinflammatory activity. Non-limitative examples of scientific communications:

Publications:

- Conde J.R., De la Fuente R., Massó J.M., Santos M. and Martorell J. "Actividad antiinflamatoria por vía oral y subcutánea de un nuevo compuesto de síntesis: Fepradinol". Rev. Farmacol. Clin. Exp. (1990) 7 : 91-98.

- Massó J.M., Conde J.R., De la Fuente R. and Martorell J., "Actividad antiinflamatoria tópica de un nuevo compuesto de síntesis: Fepradinol". Rev. Farmacol. Clin. Exp. (1990) 7 : 85-89.

- Massó J.M., Conde J.R., Villar A.M. and Martorell J., "Effect of Fepradinol on rat hind paw oedema induced by several inflammatory agents". J. Pharm. Pharmacol. Accepted for publication.

- Massó J.M., Conde J.R., Villar A.M. and Martorell J., "Mechanism of antiinflammatory action of Fepradinol". Arzneim. Forsch./Drug Res. Accepted for publication.

- Massó J.M., Villar A.M., Conde J.R. and Martorell J., "Effects of Fepradinol on rat acute models of vascular permeability and leucocyte migration". Agents and Actions. Sent for publication.

Congresses: (the titles are translated into english)

- Conde J.R., De la Fuente R. and Massó J.M. "Fepradinol. New antiinflammatory with specific activity in acute processes" XIII Reunión Nacional Asoc. Esp. Farmacol., Jaca (1984).

- Conde J.R., Massó J.M. and De la Fuente R. "Fepradinol. Antiinflammatory activity by topical route. An interpretation" XIII Reunión Nacional Asoc. Esp. Farmacol., Jaca (1984).

- De la Fuente R., Conde J.R. and Massó J.M. "Lactalfate (EL-846). Effect on gastric mucous secretion in rat. Comparison with sucralfate". X Reunión Nacional Asoc. Esp. Farmacol., Valencia (1985).

- Conde J.R., De la Fuente R., and Massó J.M. "Lactalfate (EL-846). Pepsin union capacity and antipeptic activity. Comparison with sucralfate". X Reunión Nacional Asoc. Esp. Farmacol., Valencia (1985).

- De la Fuente R., Massó J.M., Conde J.R. and Martorell J. "Fepradinol. Gastric response and effects on Central and Autonomous Nervous Systems". III Reunión Luso-Española de Farmacología (1987).

- Martínez M.P., Massó J.M., Conde J.R. and De la Fuente R. "Inactivation of the corticoid Ciclesonide by hepatic oxidases". IV Reunión Sociedad Portuguesa y Española Farmacología. Lisboa (1991).

- Massó J.M., Conde J.R., Martínez M.P. and Villar A.M. "Antiinflammatory action of Fepradinol in phospholipases A_2 and C-induced rat paw edemas". IV Reunión Sociedad Portuguesa y Española Farmacología. Lisboa (1991).

- Martínez M.P., Massó J.M., De la Fuente R., Conde J.R. and Martorell J. "A method for antihaemorrhoidal activity evaluation in rat". I Congreso Iberoamericano de Farmacología. Málaga (1992).

- Massó J.M., Conde J.R., Martínez M.P. Villar A.M. and Martorell J. "Effect of Fepradinol on vascular permeability in rat skin". I Congreso Iberoamericano de Farmacología. Málaga (1992).

3. That I am familiar with the remarks filled by Applicant in the above-identified patent application in the United States in a communication entitled "Amendment After Final", and that the data presented in those arguments are based upon experiments conducted by me and observations made by me, or under my supervision and control.

4. From a quantitative point of view, both the general pharmacological activity as well as the ratio between local and systemic effects, are related essentially to the nature of the substituent radicals and the metabolic pattern of the compounds. Relevant differences can be found between compounds containing structurally similar radicals and structure-activity relationship for the substituent is not known up to today. From the foregoing, it is clear that a higher intrinsic local anti-inflammatory activity and lower systemic glucocorticoid effect are the desirable properties for a new glucocorticoid intended for topical action, that these properties are related to the nature of the substituent groups and that is not predictable in this relation.

However, the Examiner alleges (Paper No. 6, page 6, second paragraph) that:

> **"The claimed compounds, compositions and methods of use would have obvious to one of ordinary skill in the art at the time the invention was made because the claimed compounds are structurally related compounds which would be expected to have similar chemical and pharmaceutical properties"**

In the patent of Brattsand (A) (1) mentioned by the Examiner, compounds referred in examples 1, 2 and 3 correspond to the following structures:

Example 1: (col 3, line 57)

16α,17α-(2'-Hydrogen-2'-**ethyl**) methylene dioxy-9-fluoropregna-1,4-diene-11ß,21-diol-3,20-dione

Example 2: (col 9, table in line 46)

16α,17α-(2'-Hydrogen-2'-**n-propyl**) methylene dioxy-9-fluoropregna-1,4-diene-11ß,21-diol-3,20-dione

Example 3: (col 9, table in linea 46)

16α,17α-(2'-Hydrogen-2'-**n-butyl**) methylene dioxy-9-fluoropregna-1,4-diene-11ß,21-diol-3,20-dione

So, these three compounds conform a homolog series in respect to radical R_1 of the general formula.

5. The biological effects of these compounds are summarized by Brattsand in Table 5 of his patent (col. 9) and they are reproduced in the following table (a column indicating therapeutics index values, obtained as the quotient between the ED_{50} values for systemic and topical activities, is included).

Compound	R1	Topical anti-inflammatory activity (Cotton Pellet)	Systemic glucocorticoid activity (Thymus inhib)	Therapeutic Index Systemic ED50/ Topical ED50
Ex. 1	Ethyl	35	100	2.86
Ex. 2	n-Propyl	10	> 30	> 3
Ex. 3	n-Butyl	< 3	70	> 23

From the data contained in this table, it can be seen that, in relation to the three compounds of the mentioned homolog series:

a) The ED_{50} values for topical activity are in the range <3 and 35 μg, that is, they differ by a factor of at least 10.

b) The ED_{50} values for systemic activity are in the range >30 and 100 μg, that is, they differ by a factor of 3.

c) The values for therapeutic index are in the range 2.86 and 23 μg, that is, they differ by a factor of at least 8.

6. From the data contained in the patent of Brattsand it is clear that what would have obvious to one of ordinary skill in the art at the time the invention was made than structurally related compounds would NOT be expected to have similar pharmaceutical properties.

This conclusion is of application to the compounds included in the instant patent application as well as those claimed by Brattsand in the mentioned patent.

7. The Examiner specifically have mentioned compound 16 presented in Table 4, column 5, of Brattsand and col. (A), indicating the structural similarity with the compound claimed in the instant application in which R_1 is butyl and R_2 is acetyl. While the present patent application does not contain experimental data on the pharmacological activity of said compound, the data are available in proprietary files (compound EL-854). The comparison of these data with compound 16 of Brattsand is indicated below:

Compound	Topical anti-inflammatory activity (Cotton Pellet)	Systemic glucocorticoid activity (Thymus inhib)	Therapeutic Index Systemic ED50/ Topical ED50
Ex. 16 U.S. Patent No. 3,983,233	< 3	30	> 10
EL-854	8.6	147	17.1

From the data contained in this table is derived that:

a) The ED_{50} values for topical activity differ by a factor of at least 3.

b) The ED_{50} values for systemic activity differ by a factor of 5.

8. I feel that these results do not support the allegation that the pharmacological activities of both compounds are similar, specially if the scope of minimize systemic glucocorticoid effect on claim 18 is taken in consideration.

Neither the compound indicated by the Examiner (the above mentioned EL-854) nor any other of the compounds claimed in the instant application are specifically mentioned in the patent of Brattsand et al.

9. Moreover, I feel the compounds claimed in this application shown pharmacological properties of patentable utility because there are <u>differences</u> not obvious at the time the invention was made to a person having ordinary skill in the art between the subject matter sought to be patented and the prior art.

This assessment is derived from comparison of the values of anti-inflammatory potency of the compounds from the instant invention and the corresponding values of those included in the patent of McDonald (U.S. Patent No. 4,835,145) Tables II and III (2). The results on relative potency of the compounds included in the instant invention are derived from the quotient ED_{50} (22 R,S)-compound (column "topical anti-inflammatory activity" on table III from the instant invention).

The results are showed in the following table:

Patent	Compound	Relative Topical anti-inflammatory potency (Budesonide = 1)
U.S. Patent No. 3,983,233 (Brattsand)	Ex. 22 (more potent of all the compounds	1.49*
U.S. Patent No. 4,835,145 (McDonald)	2 e 2 f 2 g 2 i	3.03 4.86 1.80 5.90
U.S. Patent Application Seriel No. 578,942 (Calatayud)	7 10 13 13	7.54 2.73 36.35 36.35

* Table III of U.S. Patent No. 4,835,145

10. The pharmacological data of the compounds of the instant invention are compared with those included in the patent of McDonald because:

a) The patent of McDonald is latter than that of Brattsand and represent the latest teaching of the relevant art.

b) Data on pharmacological activity mentioned by McDonald were acquired by the same test method employed by the instant Applicants [Method of Meier et al. (3)].

c) Budesonide is used as reference compound by McDonald and the Applicants. In this way, possible differences in experimental conditions are overcome.

From this data is derived that the compounds claimed in the present patent application shown a better anti-inflammatory potency that those included in the patents of reference.

This better response can be related to:

a) Bulkiness of the substituents, because bulk differences in R_1 can provide better responses.

b) A better topical anti-inflammatory response.

11. Moreover, I feel it is clear from the data presented in Table III of the specification that the compounds included in this application possess a better relation between the topical anti-inflammatory activity and systemic effects. This can be due to metabolic patterns of the compounds of the instant invention. Comparative studies on inactivation of Ciclesonide* by hepatic oxigenases show that the systemic action of Ciclesonide is lower than that action of Budesonide by p.o. and s.c. routes of administration. (4)

12. From the foregoing discussion, I feel it is clear that:

a) Though they are structurally related, the compounds claimed in the instant patent application are not specifically disclosed or suggested by the references.

b) They show higher topical anti-inflammatory activity (inhibition of the growth of the granuloma) and systemic effects (reduction of the thymus weight), and lower systemic effects (reduction of the thymus weight), and more advantageous hepatic metabolism; and, therefore, a better therapeutic index than the steroids of the prior art.

(*) Ciclesonide is the INN of the compound 7 included in the specifications.

13. The use of the compounds claimed in the present application are not a matter of preference depending on factors not related to pharmaceutical properties, but represents an unexpected therapeutic improvement with regard to other steroids; and, therefore, could be considered patentable.

14. The products included in the instant patent application, as well as those mentioned by Brattsand et al. contain a chiral center in C-22 and consequently they can exist as mixtures of epimers. Nevertheless, none of the epimers claimed in the present application are specifically included in or suggested by Brattsand et al. (U.S. Patent No. 3,992,534) (5).

15. Brattsand et al. (U.S. Patent No. 3,992,534) specifically mentions the compounds of their Examples 1, 3, 5, 7, 8, and 12 as preferred, based upon the characteristics of component B.

The pharmacological data of these compounds and their epimers preferred by Brattsand Table 3, columns 10 and 11, are compared with the data corresponding to instant compounds (Table III of our patent application).

Compound		µg/animal to obtain 50% inhibition of		Therapeutic Index ED50 Thymus/ ED50 Granuloma
		Granuloma growth	Thymus weight	
Ex 1*	A + B	120	270	2.25
	A	270	115	0.42
	B	30	50	1.66
Ex 3*	A + B	10	> 30	3
	A	25	> 30	1.2
	B	3	17	5.6
Ex 7*	A + B	no data	no data	-
	A	15	12	0.8
	B	10	6	0.6
Ex 8*	A + B	5	10	2
	A	6	13	2.1
	B	4	10	2.5
Ex 12*	A + B	100	80	0.8
	A	125	125	1
	B	40	70	1.75
Ex 7**	22 R,S	21.7	614.7	28.3
Ex 8**	22 S	20.5	608	29.6
Ex 9**	22 R	25.4	667.1	26.2
Ex 10**	22 R,S	59,9	583.2	9.7
Ex 11**	22 S	43	555.3	12.9
Ex 12**	22 R	74.7	592.2	7.9
Ex 13**	22 R,S	4.5	54	12
Ex 14**	22 S	3.6	49	13.6
Ex 15**	22 R	5.2	56.3	10.8

(*) Brattsand, (B) (U.S. Patent No. 3,992,534

(**) U.S. Patent Application No. 578,942

16. The following detailed tests were conducted to achieve the data of the instant invention. The experimental procedure used has been described by Meier et al (3).

The subcutaneous implantation of two cotton pellets into the axillary region of each rat, produce at on early day, the local formation of a considerable amount of new granuloma tissue around the implantation zone. According to this procedure the test substances are applied topically in the implanted cotton wads. It is thereby possible to study the local anti-inflammatory effect in granuloma and also systemic effect in the form of retrogression of thymus.

Young male rats of the Wistar strain weighing 100-120 g from ELMU, S.A. animal house were used. The animals were housed five to a cage in suspended polyethylene cages with solid floor and wooden shaving. Throughout the study each cage will be identified by a label coloured according to the group and recorded the study number, date, treatment number, level dose animal number and details of treatment.

Temperature of the animal house remain at 21 ± 2°C., the relative humidity is 50±10% and lighting will be controlled to give 12 hours light (08:00 am to 08:00 pm) and 12 hours dark per 24 hours.

Before the beginning of the study, animals were remained in acclimation for a three days interval.

Preparation of the cotton pellets.

Dental cotton rolls were cut to 2-3 mm sections, weighing exactly 20 mg each, and sterilized at 160°C. for 2 hours.

Before of its implantation, each pellet was soaked with 50 µl of the test solutions, allowing the drying of the solvent Control group will be implanted with pellets soaked with 50 µl of the solvent alone.

In the beginning of study body weight of all animals is determined under light ether anaesthesia, 2 pellets per animal will be subcutaneous implanted with adequate sterile instrument (implantation trocar), trough a small incision

of the ventral skin and passing in the direction of the two fore limbs, the pellets were deposited symmetrically in the axillary region.

In the right axillas will be implanted the pellet soaked with the test solution and in the left one, the pellet soaked with the solvent (this late pellet, is a positive control showing the development oneself experience). In the control group both pellets are exclusively soaked with the solvent.

After recovery from anaesthesia, the animals were housed again, in its respective cages, where they will remain for 7 days, avoiding during this period bruits and whichever alteration.

On the seventh day after implantation, body weight are recorded. The animals were killed with ether, and the fresh weight of thymus and adrenals was measured immediately. The pellets were dissected free as close as possible to the surface of the cotton fibbers; they were dried at 60°C. for 24 hours and weighted later.

Evaluation of results:

For evaluate the results, is measured the weight of the dried pellet and granuloma tissue developed, subtracting the weight of the implanted pellet (20 mg).

Differences of body weights between the first and seventh day of the study are determined.

The means of granuloma weight of animal of every group are calculated and standard deviation is determined.

Logarithmic regression analysis were drafted and used for estimating the doses giving 50% reduction of granuloma growth and of thymus.

The therapeutical index absolute is calculated from the ratio: ED_{50} granuloma/ED_{50} thymus.

The therapeutic index relative to Budesonide (=1) is calculated from the ratio: Therapeutical index compound/therapeutical index Budesonide.

17. From the foregoing data, it is clear that in all the cases, the therapeutic index of the compounds claimed in the instant application and their epimers are more favourable than those shown by the compounds mentioned by Brattsand et al. and component B of the afore-mentioned Brattsand compounds.

18. From the foregoing discussion, I feel that though they are broadly structurally related, the epimers claimed in the present application are neither specifically mentioned nor in any way suggested by or obvious from the teaching of the cited references; and that they show an unexpected, better relationship between local anti-inflammatory activity and systemic effects than the epimers claimed in the reference patent. I also feel that a better therapeutic index than that of the steroids of the prior art is clear, which means a significant therapeutic improvement in relation with other glucocorticoids.

The foregoing data were either developed by me, or by others working under my direct supervision and control, and the conclusions set forth herein are based upon my expert evaluation of the data presented.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or

imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued therein.

Date: 5/21/93

J.R. Conde
Director Pharmacologycal Research
Elmuquímica Farmacéutica S.L.

Cited Literature

1) Brattsand et al. U.S. Patent nº 3,983,233.

2) McDonald. U.S. Patent nº 4,835,145.

3) Meier et al Experientia 15, 469, (1950).

4) Massó J.M. et al. Reun. Soc. Port. Esp. Farmacol. Lisbon (1991).

5) Brattsand et al. U.S. Patent nº 3,992,534.